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Elevated fingernail cortisol levels in major depressive episodes

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Highlights

- Depressed patients have increased cortisol levels in comparison to controls using specimens that measure aggregate cortisol levels over a period of fifteen days.
- Increased cortisol levels may be only explained for those severe episodes with non-reactive mood and more prominent melancholic symptoms.
- Depressed patients with severe fatigue were characterised by decreased cortisol levels.

Abstract

Background:

The extent to which cortisol levels are elevated in major depressive episodes (MDE), and hence could act as a biomarker of illness, remains unclear. Although patient characteristics may explain some of this variation – for example elevated cortisol being more often found in patients with severe, psychotic or melancholic depression – problems with the methods used to measure cortisol may also have contributed to the inconsistent findings. Fingernails are a novel sample that can be used to assess aggregate cortisol concentrations over a 15-day period, and may provide a more accurate reflection of longer term cortisol level changes in MDE and help clarify this issue. This methodology has not yet been utilised in MDE.

Methods: Cortisol levels reflecting a period of 15 days were measured using fingernails in a group of 26 subjects experiencing a major depressive episode (MDE) and in an age and gender matched group of 45 healthy controls.

Results: Depressed subjects showed significantly higher mean cortisol levels measured in fingernails when compared with control subjects. Higher levels of cortisol were associated with higher depression severity scores, a diagnosis of non-reactive depression, and more prominent melancholic symptoms. Conversely, fatigue was negatively correlated with cortisol levels.

Conclusion: There is elevated cortisol in MDE when assessed using an aggregate measure

over two weeks. Alterations in fingernail cortisol correlate with key clinical symptoms and subtypes of depression.

Introduction

Hypercortisolaemia is considered a candidate biomarker potentially able to support the diagnosis or sub-typing of the major depressive disorder at biological level (Pariante 2009). However, there is some inconsistency in this finding, especially when using short-term measurements of cortisol concentrations such as salivary cortisol. This may be, in part, explained by the large heterogeneity seen within depressive syndromes. Indeed, it has been shown that depressive episodes with psychotic or melancholic features (Carroll et al., 2007, 2012; Schatzberg et al., 2002), largely account for the increased cortisol levels found in this disorder. Furthermore, this could be also due to the susceptibility of these parameters to the influence of state variables, such as the day of the week, time of day or presence of transient extraneous factors liable to alter levels such as stress (Kudielka & Wüst, 2010). Fingernails, an integumentary type of tissue that share several properties with hair (Herane-Vives et al., 2017), have recently been used and validated for measuring longer-term concentrations of cortisol (Ben Khelil et al. 2011; Izawa et al. 2015). The advantages of this methodology are manifold: it is non-invasive, economical in that repeated sampling can be avoided, and enables the assessment of cumulative cortisol concentrations over an extended period of time. To date, only one study has used this specimen in the mental health field for this purpose (Warnock et al. 2010) and none in subjects with major depression. Based on the background mentioned above, we designed this study with the objective to (1) compare fingernail cortisol concentrations in depressed subjects and matched healthy controls and (2) assess whether differences in cortisol levels are influenced by clinical variables in subjects with major depression. We hypothesised that subjects with major depressive episodes would be characterised by higher fingernail cortisol levels than healthy controls.

Methods

Participants

Participants were recruited as part of an ongoing collaboration between London (UK), Santiago (Chile) and Hong Kong. Depressed participants were recruited via public advertisements (Wise et al. 2016) and from local psychological therapy and secondary care services. Twenty-six depressed patients were recruited, twenty in London (UK), six in Santiago (Chile) and none in Hong Kong. All psychometric tools were English or validated Spanish/Chinese versions. Patients met DSM-IV criteria for a major depressive episode (MDE) in the context of either a unipolar or bipolar disorder, diagnosed using the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al. 1998) and reported no history of psychiatric illness in first degree relatives. Depression severity was assessed with the Hamilton Depression Rating Scale (HAMD-17, Hamilton 1967) with only those scoring at clinical severity (≥ 13) included (Cleare et al 2015). Rating of depressive symptoms across sites was evaluated on an independent set of patients and showed a high inter-rater reliability (Intraclass correlation coefficient=0.96, p=0.004). The Quick Inventory of Depressive Symptoms was used as a self-rated measure of depressive symptoms (Rush et al, 1993). The Young Mania Rating Scale (Young et al. 1978) was used to exclude current hypomania/mania, while historical self-reported hypomanic symptoms were assessed using the 33-item hypomania checklist (HCL-33) (Feng et al. 2016). Melancholic symptoms of depression were assessed using the Newcastle Depression Diagnostic scale (NDDS, Carney et al. 1965) and atypical depression with the Atypical Depression Diagnosis Scale (ADDS, Stewart et al 1993). This latter scale provides four categories: (1) 'non-reactive depression', (2) 'simple reactive depression', (3) 'probable atypical depression' and (4) 'a definite atypical depression'. The presence of rumination and irritability symptoms was established by using the Rumination Scale (Treynor et al. 2003) and the Self-Assessment of Irritability Scale (Snaith et al. 1978). Anxious symptoms were assessed using the "Anxiety Factor" on the 17-item HAMD-17 (Levitt et al. 1993). Environmental factors in the three months prior to study participation were assessed using the Hassles Scale (Kanner & Coyne 1981) and the Recent Life Changes Questionnaire (Miller & Rahe 1997), while early life trauma was assessed with the Childhood Trauma Questionnaire (CTQ; Bernstein et al. 1994). The presence of childhood trauma was recorded if a participant presented with a score greater than the threshold on any of the following subscales of the CTQ: emotional abuse (threshold >12),

physical abuse (threshold >9), sexual abuse (threshold >7), emotional neglect (threshold >14) and physical neglect (threshold >9). Patients were medication free for ≥2 weeks (≥4 weeks for fluoxetine) and were not receiving any psychological intervention at the time of the assessment. Healthy controls were free from current or past psychiatric diagnoses as assessed using the MINI, as were their first-degree relatives using patient history. Participants were excluded if they reported any illicit substance use in the previous three months or had any unstable medical condition. Healthy controls were free from current or past psychiatric diagnoses as assessed using the MINI, and reported no history of psychiatric illness in first degree relatives. Participants were excluded if they reported any illicit substance use in the previous three months or had any unstable medical condition. Controls (n=45) were recruited from public advertisements and from hospital and university staff across the three sites from UK (n=15), Chile (n=2) and Hong Kong (n=28). The over recruitment of controls from Hong-Kong was undertaken in order to increase the statistical power of the study. Controls were selected in order to match as closely as possible in age and gender with the depressed group. The local ethics committee approved the research and written informed consent was obtained from each participant. All participants received modest compensation for taking part in the research.

Fingernail specimens

Participants were instructed to clip their fingernails 15 days prior to the study assessment day in order to standardise the number of days' growth to be sampled (approximately 1.5 mm per nail). Subjects were provided with detailed instruction on how to cut their fingernails accurately at the desired length at the 15-day time point, store the samples correctly and post them in specific containers back to the investigators. Cortisol was subsequently extracted according to the method described by Warnock and others (2010), with minor modifications.

Fingernails extraction

Fingernail samples were washed two times with 3 ml isopropanol (LC/MS grade) in glass vials and dried overnight. Next, 20-50 mg of the washed clippings were ground (Retsch ball mill mixer; 30 Hz) and 10-25 mg of accurately weighed specimen was used for cortisol extraction (1.5 ml LC/MS methanol; 1 hour on rotary mixer). Finally, after centrifugation, 1.3

ml of the methanol supernatant was transferred to a separate tube and evaporated to dryness at 60-degree Celsius under nitrogen. The residue was redissolved in 1 ml of assay buffer and stored at -30 degree Celsius until Immunoassay (Mondelli et al. 2010). All fingernail samples were analysed in the Affective Disorders Laboratory at the Bethlem Royal Hospital, London UK.

Statistical analysis

Data were checked for normality using graphic methods, such as histograms, and the Kolmogorov-Smirnov statistical test; results indicated that the fingernail cortisol data were not normally distributed (Figure 1). Therefore, we used a non-parametric test (Mann-Whitney U test) to compare fingernail concentration between MDE participants and healthy controls and the Kruskal–Wallis test when cortisol values were compared in more than two groups. Demographic and clinical data were compared using parametric statistical tests (*t* tests) for continuous variables and *Chi*-squared for categorical variables. Finally, a regression model was created to estimate the regression coefficient (β) and 95% confidence intervals (CI) of the variance in cortisol levels in fingernails in relation to clinical and demographic continuous and categorical predictors in MDE participants. We used Generalised Linear Models (GLMs) with a gamma distribution and a log-link function to model the data so as to better take into account the right-skew in the hormone concentrations in the tissue. The GLM is a flexible generalization of ordinary linear regression that allows for response variables that have error distribution models other than a normal distribution (Nelder et al 1972). The level of significance was set at $p \leq 0.05$ (two-tailed).

Results

Detailed demographic and clinical variables of the sample are presented in Table 1. Twenty-six subjects with a MDE including one patient with bipolar disorder type I and two with bipolar disorder type II took part in the research and were sex and age matched with 45 healthy controls. Twenty depressed patients were recruited in London (UK), six in Santiago (Chile) and none in Hong Kong. None of the patients showed significant current hypomanic symptoms. Eleven subjects met criteria for atypical depression (42%) whereas 15 were

considered non-atypical (59%), based on the ADDS scale. As predicted, patients differed from controls on a number of clinical parameters including symptoms of depression and anxiety, ruminative thinking style, and early life and current environmental factors. There was no difference in the length of fingernail samples, waist circumference, or Body Mass Index (BMI) between groups.

Fingernail cortisol level in depressed subjects vs. healthy controls

Depressed subjects had significantly higher fingernail cortisol concentrations (FCC) (mean [SD]=201.2 [277.3] pg/mg, median [interquartile range, IQR]=96.4 [60.2–396.8 pg/mg]) in comparison to controls subjects using the Mann-Whitney U-test (mean [SD]=101.5 [90.5], median [IQR]=76.9 [39.2–165.6 pg/mg]; p=0.03). However, when we ran a sensitivity analysis excluding the 3 patients with bipolar disorder the difference between MDE and controls decreased to a trend level (p=0.09). There was no significant difference in FCC between different sites. FCC values for UK depressed participants were: mean [SD]=176.4 [235.9] pg/mg, median [IQR]=94.1 [70.1–154.8 pg/mg] and for Chilean depressed subjects were: mean [SD]=193.6 [268.3], median [IQR]=96.4 [77.5–160.8 pg/mg]; p=0.27 using the Mann-Whitney U-test. FCC values for control subjects were: UK: mean [SD]=142.6 [194.8] pg/mg, median [IQR]=88.8 [62.9–121.3 pg/mg], Chile: mean [SD]=100.0 [85.3], median [IQR]=84.6 [62.0–105.3 pg/mg]; and Hong-Kong: mean [SD]=101.5 [90.5], median [IQR]=76.9 [62.0–105.4 pg/mg]; p=0.29 using the Kruskal–Wallis -test.

Regression analyses of fingernail cortisol levels with clinical variables

The generalised linear model showed that higher levels of cortisol were associated with higher depression severity scores, a diagnosis of non-reactive depression, and more prominent melancholic symptoms (all $p<0.05$; Table 2). Conversely, lower levels of cortisol were associated with more severe fatigue ($p<0.05$; Table 2). No other clinical variables exerted a significant effect on cortisol levels in comparison to healthy participants (all $p > 0.05$).

Discussion

In this study we set out to measure differences in cortisol levels in MDE by using a novel

technique utilising fingernails, representing aggregate cortisol concentrations averaged over a period of 15 days. We demonstrated that fingernail cortisol concentration is increased overall in MDE compared with healthy controls. The GLM also showed that higher levels of cortisol were associated with higher depression severity scores, features of non-atypical depression, and more prominent melancholic symptoms. Conversely, more severe fatigue was associated with decreased cortisol levels.

Medium-term cortisol levels

These results suggest that overall cortisol levels are elevated during an MDE when assessed cumulatively over a 15-day period. This provides additional evidence over and above findings of elevated shorter-term measures of cortisol such as blood, saliva and urine, and strengthens the position of elevated cortisol release as an important neurobiological correlate of depression (Pariante 2009). Further support for the elevation of cortisol over longer time frames comes from studies using hair specimens from depressed patients, which usually cover longer periods of around 3 months (Dettenborn et al. 2012; Herane Vives et al 2016, Wei et al. 2015, Pochigaeva, 2017).

Clarification that raised cortisol is a persistent phenomenon, particularly in those with more severe forms of depression with typical features, suggests that some patients with depression manifest the same neurobiological disturbance as Cushing's syndrome, but in a less severe form. Chronic hypercortisolism in Cushing syndrome has a variety of potentially deleterious effects, including obesity, hypertension, glucose intolerance, osteoporosis, impaired immune function, and poor wound healing (Pennacchietti 2015). However, the degree of hypercortisolism, when present in depression, is of a milder form compared to that Cushing's syndrome. For example, one study found a median fingernail cortisol of 679 pg/mg in Cushing's syndrome (Thomson et al. 2010), which is much higher than the median of 96.4 pg/mg in the current study, notwithstanding that direct comparison is hampered by some differences in the techniques used. Nevertheless, several depressed individuals did have fingernail cortisol levels in the 200-1000 pg/mg range (see Fig 1) which are more similar to those seen in Cushing's syndrome. Overall, however, the level of cortisol measured in depressed subjects appears to fall within the range described as subclinical hypercortisolism (Dalmazi & Pasquali 2015). This condition has to date been generally been applied to

subjects with 'adrenal incidentalomas' and not from depression. Given the potential impact of chronically elevated cortisol levels as demonstrated in fingernails, depression ought perhaps to be included within the remit of subclinical hypercortisolism.

The occurrence of depressive recurrences would be expected to lead to repeated, periodical high level of cortisol (Mueller & Leon 1999). This could potentially contribute to factors such as poorly controlled blood pressure (Bednarek & Jankowski 2014), raised glucose levels (Gillmer et al. 1975) and other more complex cardiovascular or metabolic condition often coexisting in subjects with subclinical hypercortisolism (Dalmazi & Pasquali, 2015) and depression (Katon, 2003). Another possible correlate of elevated cortisol is that of persistent cognitive impairment, which is present in some depressed patients and has been found to affect most of the cognitive domains (Porter et al. 2003). If the impaired cognition in patients with histories of more severe depression is linked to hypercortisolism, this raises the possibility that drugs such as mifepristone, a specific glucocorticoid receptor blocker, may have a therapeutic benefit in this neurocognitive impairment (Young et al. 2004).

Relation to depression sub-type

We found that higher fingernail cortisol was associated with more severe depression, and with depressive episodes characterised by melancholic and non-reactive mood of depression. This supports previous research suggesting that Hypothalamic–Pituitary–Adrenal (HPA) axis hyperactivity is more prominent in melancholic (Carroll & Feinberg 1981) and psychotic (Schatzberg et al. 2002) sub-types of depression. Therefore, elevated cortisol levels may be a potentially relevant biomarker particularly for those particular forms of depression.

On the other hand, we did not find that fingernail cortisol was specifically linked to sub-type of atypical depression as the degree of broadly defined by the ADDS atypical symptomatology. Atypical depression has elsewhere been described to have lowered rather than elevated cortisol levels (Gold & Chrousos 2002). These previous studies assessed only short-term cortisol levels (Lamers et al. 2013; Gold & Chrousos 1999), and it is possible that hypocortisolaemia is not a chronic phenomenon in atypical depression.

However, we did find that one of the features typically associated with atypical depression, severe fatigue, was negatively correlated with cortisol levels. Our results, therefore, provide

new evidence that indicates that this somatic symptom is linked to lowered cortisol levels, not only in other disorders which have commonly been associated with low cortisol levels, such as Chronic Fatigue Syndrome (Cleare 2003), but also in affective disorders, such as MDE. It has been suggested that lowered cortisol may be an aetiological factor in CFS (Cleare 2004), given that low dose cortisol replacement can alleviate the experience of fatigue (Cleare et al 1999), which raises similar possibilities in patients with fatigue within affective disorders (Cleare 2009).

Methods for assessing longer-term cortisol levels

Cortisol measurement from fingernails presents a number of advantages over hair extraction, the other novel specimen recently introduced for measuring chronic cortisol levels over longer periods. Specific confounding factors such as cosmetic treatment and frequency of washing for hair samples have not been found in fingernails, rendering results less susceptible to these extraneous factors, and facilitation easier matching of subject characteristics in case-control studies. Frequently, male subjects cannot be included in research using hair specimens because they are more likely to have a hair length shorter than three centimetres, the standard measure that these studies use (Dettenborn et al. 2010). Moreover, there remains a lack of agreement as to the optimal way to analyse hair samples (Pragst & Balikova 2006). Whilst some studies have ground hair before extracting cortisol, others have only cut them in small pieces (Davenport et al. 2006). The procedure of hair sampling can also have an aesthetic effect, leading potential subjects to avoid providing hair samples (Izawa et al. 2015). In a recent community study, we found that 42% of subjects were unable to give hair due to shortness or baldness, while another 29% refused to give hair for various reasons (Fischer et al, 2016).

Limitations

There are some methodological issues in this study worthy of discussion. First of all, a more complete description of cortisol changes in depression could have been achieved by including another specimen alongside fingernails. This would have revealed any differences in cortisol levels in this group of patients assessed across different time periods. Nevertheless, we

believe that the results using fingernails stand on their own and are an already validated method for measuring stable cortisol levels. Thus, fingernails have been demonstrated to be unaffected by acute influences (Izawa et al. 2015), a key requirement in order to reflect stable cortisol concentrations. Moreover, the use of fingernail cortisol has been validated in one recent study (Izawa et al. 2015) that found a positive and significant association between fingernail and hair cortisol levels – the latter being itself a now well-validated specimen for quantifying stable cortisol concentrations (D'Anna-Hernandez et al. 2011; Sauvé et al. 2007; Dettenborn et al. 2010). Finally, the fact that fingernails share the same keratinized features as hair (Herane-Vives et al. 2017) provides a theoretical underpinning of the results of Izawa et al (2015) suggesting that both specimens are likely to be reliable measures of chronic cortisol concentrations. Nevertheless, we suggest that further larger studies comparing these two specimens may help reinforce these findings.

We must also acknowledge that our recruitment of patients and controls was not evenly balanced between centres, introducing potential site heterogeneity and ethnic variation. Whilst there is no evidence to date that fingernail cortisol differs between Asian and European or Latin cohorts, and we found no difference in our groups by centre of recruitment, an examination of ethnic differences was not a primary goal of the study. Thus, it will be important for future studies to explicitly test whether there are subtle effects of ethnicity on fingernail cortisol measures, and for future studies of fingernail cortisol in depression to try to recruit a more homogeneous sample than we have done in order to eliminate possible confounding effects of centre or ethnicity.

It is not clear why two specimens that have been validated for measuring chronic cortisol levels provide different levels of cortisol concentration. Indeed, several studies have detected an increased cortisol concentration in fingernails in comparison to hair samples (Raul et al. 2004; Ben Khelil et al. 2011), suggesting that any potential *wash-out effect in fingernails* has a smaller impact on cortisol concentrations than in hair. Nevertheless, the actual magnitude of the potential wash-out effect in cortisol fingernail levels has not yet been explicitly studied. Wash out effects do play a clear role in diminishing cortisol concentration above the 3rd to 4th cm of hair (Kirschbaum et al. 2009). However, the average period of time assessed using fingernails in this study was equivalent to 0.61 cm of hair. Although it seems unlikely that there will have been a large washout effect in such a time period, we cannot definitively

exclude this as it has not been studied for such short periods of time/short lengths of hair. It is possible, therefore, that there is an additional early wash-out effect on the first millimetres of hair as soon as this specimen leaves the epidermis, after which cortisol concentrations then remains stable up to the three cm point, after which time other factors – such as UV radiation, shampoo or dye – exert an effect and decrease cortisol levels in hair.

Another possible factor of relevance relates to the expression of hydroxysteroid (11-beta) dehydrogenase 2 (HSD-2), the enzyme that converts either cortisol to cortisone or cortisone to cortisol. In fact, while several studies have found that cortisone concentrations are similar in fingernails and hair (Raul et al. 2004; Ben Khelil et al. 2011), cortisol concentrations were shown to be higher in fingernails (Ben Khelil et al. 2011) than in hair (Raul et al. 2004). It is possible, therefore, that hair-associated tissues, such as sweat glands (Raul et al. 2004) express larger amounts of HSD-2 in comparison to nail-associated tissues (e.g. the nail cuticle) leading to a differential cortisol/cortisone ratio in those tissues.

However, this may prove to be less problematic than might first appear. Hair follicles are closely associated with two glands – the sebaceous gland and the apocrine gland. In scalp, the hair follicle and the sebaceous gland are fused both anatomically and functionally into what is called a pilosebaceous unit. But while the ducts of apocrine and sebaceous glands empty into the follicle, the ducts of eccrine glands, which are located near the follicles, do not empty into these structures but instead directly onto the skin surface. It means that, regardless of the amount of cortisol that these glands may deliver, this should be removed if this specimen is pre-washed with methanol before measuring cortisol inside the shaft, which is the cortisol which represents the systemic values (Grass et al. 2015).

Another concern would be if the other potential sources of cortisol in hair, such as apocrine and sebaceous glands, overexpressed 11-β-HSD-2 in scalp relative to other tissues. However, we are not aware of any evidence that this is the case. Thus, it does not seem likely that differences in cortisol metabolising enzymes account for the observed differences in the amount of cortisol detected between hair and nail specimens. However, it seems more likely that other variables, such as differences in those mechanisms used by cortisol to cross cell membranes, may provide an explanation.

First of all, it is important to highlight that for nails, in contrast to hair, no glandular elements

are found in the cuticle or its surrounding tissues. Therefore, all cortisol accumulated in nails comes from the systemic circulation. In relation to this point, it has been shown that several lipophilic substances such as lead and arsenic among others, are deposited in nail after diffusing passively form blood (de Berker et al. 2007). Therefore, cortisol, being a lipophilic hormone, should not be an exception. This mechanism of transportation is observed when substances move across an area of higher concentration to lower concentration. This means that cortisol would diffuse more easily when its systemic value is high. These episodes can be clearly found in two circumstances: in generalised hypercortisolemic status, such as a primary Cushing's syndrome or in some subtypes of depression, or during periods when momentary increases of cortisol occur, such as those seen during early mornings. Thus, the physiology and histology of nails suggest that this specimen, instead of simply averaging daily cortisol outputs, as hair does, averages peaks of cortisol, at least in healthy people. In other words, nails preferentially accumulate cortisol when its concentration is high to freely diffuse from the systemic circulation. If this is the case, then our results may indicate that depressed outpatients have an increased medium-term cortisol reactivity levels in comparison to healthy people.

In this article, we referred to the period of time over which cortisol is accumulated by fingernails as "medium-term". We use this term since it is clear that we need to differentiate measures of acute cortisol levels, such as blood and saliva, from measures of chronic or averaged cortisol levels, such as hair and fingernails. However, it is also clear that the time-frame covered by hair and fingernails differs; hair cortisol most commonly uses 3 cm of hair to represent the previous three months and fingernails (e.g. Warnock et al. 2010) the previous 15 days, as we used here. We wanted to choose a term that best encompasses this difference, and thus suggest using "medium-term" and "long-term". We do consider that future research studies into cortisol changes in depression should focus on using short-, medium- and long-term specimens, and that these may indeed measure different but complementary aspects of the disorder.

Some limitations in the use of fingernails include possible effects on cortisol levels induced by nail growth rate, which can vary with seasonal changes, sex, different finger digits, clipping frequency, nail filing, and nail-biting habits (Gupta et al. 2005), although common cosmetic products such as nail varnish use are unlikely to affect fingernail cortisol (Ben

Khelil et al. 2011). Barker and others (2007) suggested that the length of fingernails is very important in relation to the concentration of cortisol extracted over a specific period of time. However, there were no differences in the length of fingernails between cases and controls in our sample, which indicates that the period covered by the nail specimens in this study was comparable between cases and controls.

Future, larger studies using fingernails could compare more directly the degree of hypercortisolemia that depressed subjects present in comparison to subjects with clearly established hypercortisolism, such as those with Cushing's syndrome, and link this to the presence of adverse metabolic and other physical effects. Studies using fingernails could also investigate cortisol levels in other specific non-atypical subtypes of depression, such as those with DSM specifiers such as anxious-distress, melancholic or psychotic features. Future studies might also investigate whether chronic hypercortisolemia in fingernails is a specific finding of unipolar or bipolar depression; given the small number of MDE patients with longitudinal diagnoses of bipolar disorder in the current study we have been unable to do this. This analysis seems particularly important since the exclusion of the small number of patients with bipolar disorder decreased the difference in cortisol levels compared to controls from a significant to a trend level. Longitudinal studies may also be helpful in validating the pattern of cortisol secretion in relation to other features of depressive illness such as remission, relapse, chronicity and comorbidity. Prospective cohort studies in young people with depression could evaluate whether cortisol may play a causative role in the association between medical conditions, such as diabetes and heart disease, and depression.

Conclusions

The use of fingernails for measuring cortisol levels aggregated over 15 days showed that depressed outpatients as a whole have elevated medium-term cortisol levels in comparison to control subjects. The present results also suggest that patients with more severe depression, and with non-reactive and melancholic features, are particularly at risk of elevated cortisol, whereas levels may be relatively lower in patients with marked fatigue. The demonstration that cortisol levels may be elevated over a longer time frame than previous research suggests that some depressive episodes may be part of a group of conditions that have subclinical hypercortisolaemia. The potential role of these longer-term elevations of cortisol in medical

conditions that occur at higher rates in depressed subjects needs to be further investigated.

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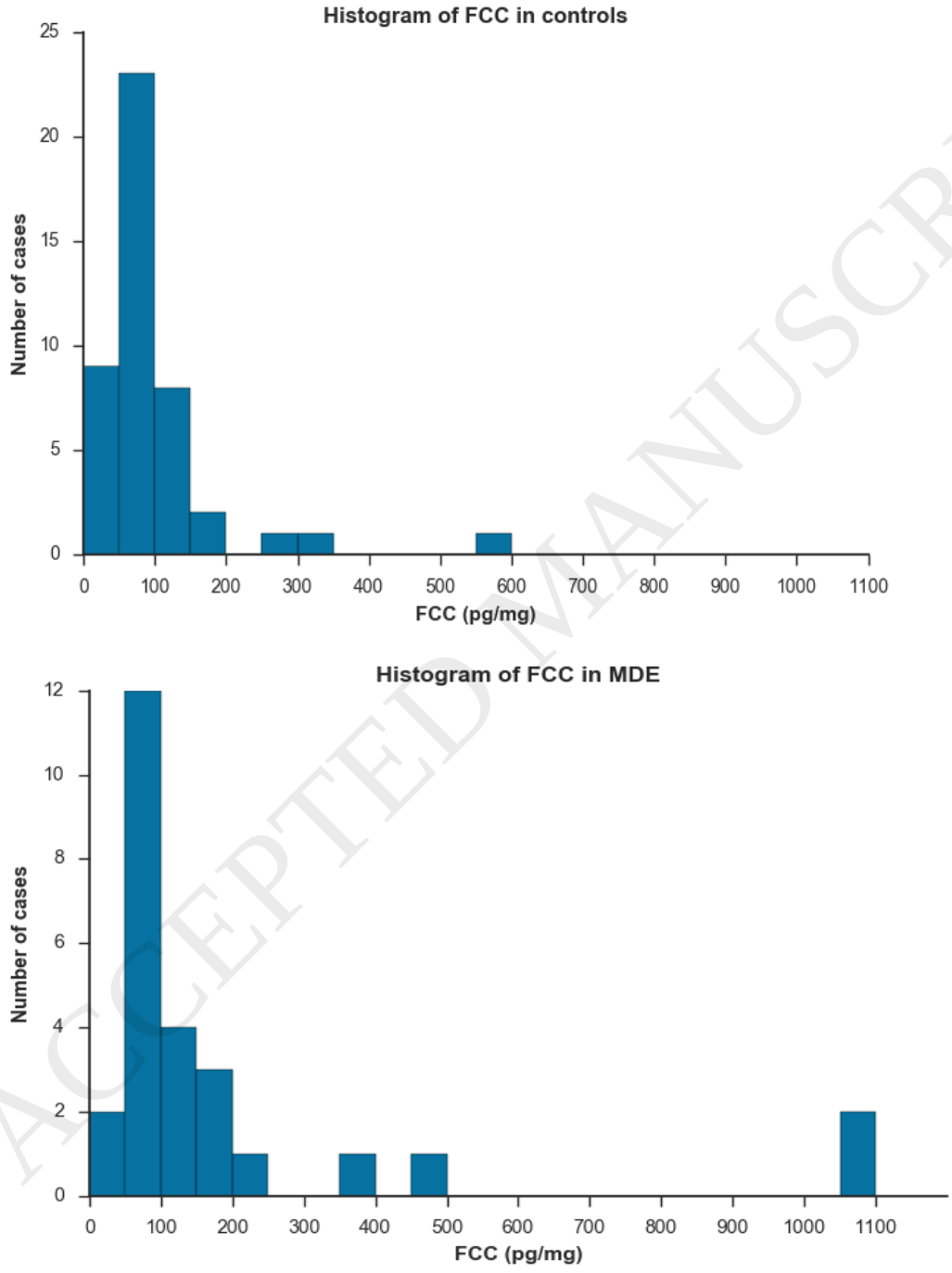
Figure caption

Figure 1: Distribution of Fingernail cortisol concentration (FCC) in controls and MDE participants



Data were significantly skewed ($p<0.05$) according to the Kolmogorov-Smirnov test.

Table 1: Demographic and clinical characteristics of the sample

Demographic and Clinical Variables	Healthy Controls	Depressed Subjects	P value[a]
N (F/M)	45 (24/21)	26 (19/7)	0.1
Age (Years), Mean (SD)	39.0 (10.3)	38.1 (12.1)	0.73
Number of previous episodes, Mean (SD)	0 (0)	2.6 (3.81)	<0.01
Duration of current episode (weeks), Mean (SD)	0 (0)	90.2 (133.5)	<0.01
Number of admissions, Mean (SD)	0 (0)	0.17 (0.54)	<0.01
HAMD-17, Mean (SD)	0.4 (0.8)	19.0 (4.4)	<0.01
Anxiety Factor, Mean (SD)	0 (0)	5.8 (3.1)	<0.01
QIDS, Mean (SD)	0.4 (0.9)	17.9 (3.6)	<0.01
YMRS, Mean (SD)	0 (0)	0 (0)	1
HCL-33, Mean (SD)	12.5 (8.0)	17.7 (5.4)	0.01
NDDS, Mean (SD)	0 (0)	3.3 (2.4)	<0.01
History of childhood Trauma (CTQ), N (%)	3 (17.7)	18 (69.2)	<0.01
SAI, Mean (SD)	6.6 (4.7)	26.3 (7.2)	<0.01
Ruminations scale, Mean (SD)	31.0 (12.2)	89.3 (77.4)	<0.01
Life events score (RLCQ), Mean (SD)	114.5 (227.6)	384.0 (321.5)	<0.01
History of severe life events (RLCQ) (last 3 months), N (%)	3 (17.7)	20 (76.9)	<0.01
Number of Hassles (last month), Mean (SD)	22.3 (26.3)	100.0 (95.9)	<0.01
Number of Severe Hassles (last month), N (%)	1 (5.9)	11 (42.3)	0.01
Length of fingernails (mm), Mean (SD)	1.6 (0.5)	2.0 (0.9)	0.09
Waist circumference (cm), Mean (SD)	83.2 (12.0)	89.3 (16.0)	0.18
BMI (Kg/m^2), Mean (SD)	24.8 (3.7)	26.9 (6.0)	0.21

[a]: t-test for continuous variable, Chi-squared for categorical variables. HAMD-17: 17-item Hamilton Depression Rating Scale; QIDS: 16-ite, Quick Inventory of Depressive Symptoms; NDDS: Newcastle

Depression Diagnostic Scale; HCL-33: 33-item Hypomania Checklist; SAI: Self Assessment of Irritability Scale; CTQ: Childhood trauma questionnaire; RLCQ: Recent Life Change Questionnaire; YMRS: Young Mania Rating Scale; BMI: Body Mass Index.

Table 2: Generalised linear model analysis between illness variables and fingernail cortisol concentration in MDE participants

Variable	Crude			Adjusted		
	β	P value	CI	β	P value	CI
Illness onset (years)	-0.02	0.25	-0.05; 0.01	0.02	0.38	-0.05; 0.02
Number past episodes	-0.10	0.37	-0.07; 0.02	-0.01	0.59	-0.05; 0.03
Number past admissions	-0.02	0.89	-1.49; 1.29	0.09	0.88	-1.02; 1.19
Duration of the episode (weeks)	<0.01	0.98	0.01; 0.01	<-0.01	0.73	<-0.01; <0.01
Severity of depression	0.09	0.02*	0.01; 0.16	0.05	0.20	-0.03; 0.13
Fatigue symptoms	-0.05	0.26	-0.14; 0.03	-0.04	*0.04	-0.09; <-0.01
Anxiety symptoms	0.11	0.05	<-0.01; 0.22	0.06	0.32	-0.06; 0.18
Melancholic symptoms	0.19	0.01*	0.03; 0.34	0.12	0.17	-0.05; 0.31
Non-reactive depression∫	0.90	0.01*	0.2; 1.6	0.88	0.02*	0.08; 1.67
Simple reactive depression∫	-0.26	0.65	-1.42; 0.89	-0.23	0.75	-1.67; 1.21
Probable atypical depression∫	-0.28	0.50	-1.11; 0.55	-0.25	0.57	-1.14; 0.63
Definitive atypical depression∫	-0.15	0.76	-1.19; 0.87	-0.14	0.79	-1.22; 0.93
Irritability symptoms	-0.05	0.10	-0.12; 0.01	-0.03	0.33	-0.10; 0.03
Rumination symptoms	<-0.01	0.28	<-0.01; <0.01	<-0.01	0.64	<-0.01; <0.01
Hypomanic symptoms	<-0.03	0.65	-0.18; 0.11	<-0.01	0.92	-0.10; 0.09
Childhood trauma	-0.45	0.44	-1.60; 0.70	-0.23	0.62	-1.17; 0.70
Daily hassles	<-0.01	0.65	-0.02; 0.01	<-0.01	0.87	-0.01; 0.01
Recent Life events	<-0.01	0.06	<-0.01; <0.01	<-0.01	0.23	<-0.01; <0.01

The β was calculated using GLM with a gamma distribution and a log-link function. Fingernail cortisol analysis was adjusted for age and gender. * P value significant at 0.05 and CI does not cross zero. CI: 95% Confidence interval. ∫: subtypes of depression based on ADDS scale